

TOBACCO INDUSTRY RESEARCH COMMITTEE  
350 FIFTH AVENUE NEW YORK 1, N. Y.

**6. Budget Plan:**

Salaries	Expendable Supplies	Application For Research Grant
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Overhead

Other

#128

Date:

March 14, 1956

\$10% on all items except personnel and grants.

**7. Name of Investigator:**

1. Paul S. Larson, Ph. D.
2. H. B. Haag, M.D.
3. Herbert McKennis, Ph.D.

**8. Title:**

1. Professor of Pharmacology
2. Professor of Pharmacology
3. Professor of Research Pharmacology

**9. Institution, City, State:** University of Virginia Medical School, Charlottesville, Virginia  
**10. Address:** University of Virginia Medical School, Charlottesville, Virginia

**11. Project or Subject:**

Enzymatic Transformations of Nicotine

**12. Additional Requirements:**

That, if any funds are still dependent on this project, they are

**13. Detailed Plan of Procedure (Use reverse side if additional space is needed)**

**14. Additional Information (including relatives of other projects and other sources of supply):**

Numerous experiments on animals have shown that only a small percent of administered nicotine is excreted unchanged (Larson, Ind. Eng. Chem. 44, 279, 1952). However, studies with C<sup>14</sup> labeled nicotine show that virtually all of the C<sup>14</sup> is excreted in the urine within 36 hours (Ganz, Kelsey and Galling, J. Pharmacol. & Exper. Therap. 103, 209, 1951; Bennett, Tedeschi & Larson, Arch. Int. pharmacodyn. 98, 221, 1954). Recent studies in this laboratory on urine from dogs receiving C<sup>14</sup> labeled nicotine indicate the presence of three major and possibly four minor metabolites of nicotine (Owen & Larson, Fed. Proceedings 14, 376, 1955). The chemical nature of these biotransformation products is currently being investigated.

To complement these *in vivo* studies on nicotine metabolism, it is proposed to initiate *in vitro* studies directed toward elucidating the enzyme systems involved. Since early studies in which tissue slices were used, no investigations on the enzymatic transformation of nicotine by animals have been recorded (Weiss et al., Biochem. Z. 308, 355, 1941; ibid, 312, 531, 1942; Annal. d. Chem. 562, 60, 1949). These studies provide little information other than a few characteristics of the systems involved.

Data on possible intermediates in the metabolism of nicotine being obtained in our laboratory, together with general advances in enzymology, make possible a study in systems less complex than whole tissue slices. For example, purified preparations fortified with co-factors according to modern enzymatic techniques should yield new information. The fact that possible intermediates have been disclosed also makes possible new studies on the effect of oxidation - reduction and other type enzymes on these compounds. Results from the enzymatic studies would be compared to intact animal studies using various species. C<sup>14</sup> labeled nicotine will be used in experiments where such labeling is desirable.

/f. L. Tuckins, Clappeller  
Business Officer of the Institution

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## 6. Budget Plan:

Salaries	\$13,000
Expendable Supplies	4,000
Permanent Equipment	10,050
Overhead	1,730
Other	300
Total	\$29,080

\*10% on all items excepting permanent equipment.

7. Anticipated Duration of Work: Paul S. Larson, Ph.D.  
Dr. Herbert McKennis, Ph.D.

P. H. S. Haag, M.D.

Three years

8. Facilities and Staff Available: Professor of Pharmacology      Dr. Professor of Pharmacology

General pharmacologic and biochemical equipment and laboratory. Landy-type Warburg apparatus, refrigerated centrifuge, C14 counting equipment (Libby anticoincidence counter, Nuclear Instruments gas-flow counter with automatic sample changer), paper chromatographic equipment and electrolytic desalter, greenhouse facilities for growing C14 labeled plants

4. Project or Sub-project: Part-time services of - Dr. Paul S. Larson

Associate Professor of Biochemistry of Nicotine Dr. H. S. Haag

Dr. Herbert McKennis

9. Additional Requirements:

What, if any, there may be will depend on how the project develops.

10. Additional Information (including relation of work to other projects and other sources of supply):

The breakdown of permanent equipment items is as follows: Perkin Elmer \$13,500, Spectracord 4000 with attachments \$8400, thermocap \$78, circulating pump \$42, Senior Isotemp Oven \$240, International Centrifuge Size No. 2 with attachments \$960, Welch vacuum pump 1402 USD with guard \$310. The major item of the Spectracord is needed for reaction rate studies, identification studies, etc., in this and in the other programs underway in this laboratory involving tobacco and its constituents.

The salary item is intended for stipends for two additional professional scientists to be added to the staff, one at the M.S. level (\$5,500), the other at the Ph.D. level (\$7,500).

In view of the need from the start of this project for the permanent equipment items, it is requested that if this application is granted that the initial payment will be for \$15,000. [Signature: Dr. Paul S. Larson, M.D., 1948; Dr. H. S. Haag, M.D., 1948; Dr. Herbert McKennis, Jr., M.D., 1948]. The following provides little information other than a few characteristics of the systems involved.

Data on tobacco intermediates in the metabolism of nicotine being obtained in our laboratory, together with general advances in the possible study of tobacco leaf complex, whole tissue slices, /s/ Paul S. Larson, possible preparation of tobacco with co-fractionating materials such as cellulose, /s/ H. S. Haag, possible new techniques, the last mentioned being a Director of Project possibility of synthesis of the aliphatic and other type enzymes on these backgrounds. Results from the cigarette studies would be expected to intact animal studies using radioactive C14-labeled nicotine will be used in experiments where such labeling is indicated. /s/ W. F. Tompkins, Comptroller

Business Officer of the Institution

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